UNITED STATES DISTRICT COURT

DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff, v. HOECHST MARION ROUSSEL, INC. and TRANSKARYOTIC THERAPIES, INC., Defendants.	Civil Action No. 97-10814-WGY CONFIDENTIAL SUBJECT TO PROTECTIVE ORDER FILED UNDER SEAL

DECLARATION OF RICHARD D. KOLODNER, PH.D.

I, RICHARD D. KOLODNER, declare as follows:

I. Qualifications.

1. I am a Professor of Medicine, Member of the Cellular and Molecular Medicine Program at the University of California, San Diego School of Medicine, and Head of the UCSD Cancer Center/Cancer Genetics Program. I am a Member of the Ludwig Institute for Cancer Research and Head of the Laboratory of Cancer Genetics at the Ludwig Institute San Diego Branch. I received a B.S. and Ph.D. in Biological Sciences from the University of California, Irvine, and was a postdoctoral fellow at Harvard Medical School from 1975-1978. From 1978 to 1997, I was a Professor at Harvard Medical School in the Department of Biological Chemistry and Molecular Pharmacology and the Dana-Farber Cancer Institute Department of Cancer Biology. I have conducted research in the fields of molecular biology and biochemistry since 1971.

2. Since 1978, I have studied the mechanism of homologous recombination and DNA repair, encompassing studies in bacteria, yeast, and mammalian cells. In 1983-1984, I was actively engaged in studies of homologous recombination in bacteria and yeast. In 1996, I received the Charles S. Mott Prize. I have been an associate editor of the journals Cell and Cancer Research since 1995 and 1996, respectively. I have served on the editorial board of Molecular and Cellular Biology since 1999. I have co-authored more than 150 scientific articles. Attached as Exhibit A is a copy of my curriculum vitae.

3. I understand that Defendants have filed a contingent motion for summary judgment that Claims 1 and 3 of the '349 Patent, Claims 6-8 of the '698 Patent and Claim 1 of the '422 Patent ("the Claims at Issue") are invalid for lack of written description and enablement. My declaration is submitted in support of Amgen's Opposition to Defendants' Contingent

Motion.

4. In forming the opinions expressed here, I have reviewed the Amgen '933 Patent and the claims of the '422, '698 and '349 Patents, the parties' respective Claim Construction Submissions, Defendants' Contingent Motion for Summary Judgment filed November 23, 1999, and accompanying papers, and the documents attached as Exhibits.

II. Summary of Opinions.

5. I have been asked to assess whether one of ordinary skill in the field of molecular biology at the time of Amgen's inventions, would have understood from reading Amgen's patent that Dr. Lin possessed the subject matter of the Claims at Issue. I also have been asked to assess whether one of skill in the art could have practiced the Amgen inventions using routine experimentation.

6. My opinions are summarized below:

Opinion 1. A molecular biologist at the time Dr. Lin filed his applications would not have considered Dr. Lin's inventions limited to uses of "cloned" "exogenous" EPO DNA because there is no scientifically significant distinction between "cloned" "exogenous" EPO DNA and "endogenous" EPO DNA.

Opinion 2. Defendants' method of producing human EPO is neither "pioneering" nor "third-wave," but rather a logical implementation of Dr. Lin's inventions.

Opinion 3. Defendants could not have achieved expression of human EPO in human cells without using Dr. Lin's inventions.

Opinion 4. A molecular biologist at the time of invention could have made and used Dr. Lin's inventions using routine experimentation.

III. Homologous Recombination Background.

7. Using homologous recombination techniques, molecular biologists can make two DNA molecules, having identical or nearly identical sequences, recombine to form two new hybrid molecules. The key requirement for this process is that the two stretches of DNA have sequences that are identical or nearly identical, i.e. "homologous." Homologous recombination

involves the exchange of a DNA sequence from one DNA strand to another, resulting in formation of two new recombinant DNA molecules.[1] If two DNA strands are not sufficiently homologous, they will not recombine.[2]

8. Homologous recombination techniques can be used to recombine two chromosomal DNA molecules or two non-chromosomal DNA molecules having identical or nearly identical sequences that are inserted into a mammalian cell. The same basic techniques can be used to alter a chromosomal DNA sequence in a cell by recombination with a non-chromosomal DNA sequence that is introduced into the cell. This latter use of homologous recombination is sometimes referred to as "gene targeting." This type of homologous recombination involves a series of recombinant DNA techniques, ultimately resulting in a DNA sequence inserted in a chromosome to form a recombinant cell.

9. To achieve "gene targeting" using homologous recombination techniques, one must know sufficient DNA sequence of the gene to be targeted and the surrounding DNA. Without such knowledge, one cannot design or select the inserted DNA so that it will recombine into the desired chromosomal locus.

10. Like PCR (polymerase chain reaction) and automated DNA sequencing, homologous recombination is simply another tool for manipulating DNA to create recombinant DNA and recombinant cells useful in producing recombinant proteins.

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[1] See Exh. B (diagram of homologously recombined DNA molecules).

[2] See Rubnitz and Subramani, Mol. Cell. Biol. 4:2253-58 (1984) (Exh. C).

Opinion 1. A molecular biologist at the time of invention would not have considered Dr. Lin's inventions to be limited to uses of "exogenous" EPO DNA because there is no scientifically significant distinction between "cloned exogenous EPO DNA" and "endogenous EPO DNA."

11. Defendants attempt to create an artificial distinction between what they call a "cloned exogenous" EPO DNA and an "endogenous" or "native" EPO DNA. The terms "cloned," "exogenous" and "endogenous" are merely labels Defendants use to describe the same DNA sequence, i.e., the natural human EPO gene. Defendants' focus on the location of the DNA sequence, i.e., whether it is a "cloned" piece of DNA or whether it is residing in the chromosome, is a distinction without scientific significance because the true utility of the information of any piece of DNA is not its location, but its sequence and organizational structure.

12. A molecular biologist at the time of invention would have understood that Dr. Lin's Figure 6 DNA sequence is the sequence of the "endogenous" human EPO gene that naturally resides in human cells. When Dr. Lin "cloned" the human EPO gene, he isolated it from a collection (or "library") of DNA sequences taken from the natural chromosomes of a human cell. One of the many advantages of having the cloned human EPO gene is that it permits characterizing its structure and function. But, that DNA sequence is still the same structure that exists naturally within human cells.

13. Because the detailed structural information provided by Dr. Lin describes both the "endogenous" and "exogenous" human EPO gene, scientifically, the distinction in terms of its location is unimportant. The true significance of what Dr. Lin achieved by cloning the human genomic EPO gene is that he provided an accurate description of its sequence and structure, which made possible for the first time, production of therapeutic EPO, cells capable of producing therapeutic EPO, and numerous other materials and methods involving the human EPO protein

and gene. A molecular biologist would consider the sequence and structure the critical information, and not limit the inventions just to uses of a "cloned" piece of DNA.

14. When one clones a DNA containing a gene, one does not envision using that gene in only its cloned form. For example, a human geneticist would clearly contemplate using the gene to study allelic variants or disease-causing mutations within cells from many individuals. The structure of the gene and the protein it encodes permits a vast number of applications, including diagnostics, transgenic animals, and mapping the gene and other nearby genes, just to name a few. There are many applications of the inventions disclosed in Dr. Lin's patents, which a molecular biologist would not have considered limited to using only the cloned version of the gene.

15. Reading Dr. Lin's patent, a molecular biologist would understand that Dr. Lin's inventions were clearly not limited to only uses of an "exogenous cloned EPO DNA sequence." For example, manufacturing synthetic EPO polypeptides[3] does not require use of an "exogenous," cloned DNA sequence, only knowledge of the amino acid sequence of the desired protein or polypeptide. Preparation of monoclonal antibodies to EPO polypeptides or to recombinant human EPO[4] also does not require use of the cloned DNA sequence, only use of recombinant or synthetic human EPO or fragments thereof. Synthetic DNA sequences encoding human EPO[5] can be prepared based on the amino acid sequence of human EPO, which before Dr. Lin's work was never possessed by any researchers. Similarly, a molecular biologist would have understood that the pharmaceutical human EPO purified from mammalian cells in culture, methods of producing human EPO, and vertebrate cells capable of producing human EPO in

[3] See '933 Patent, Col. 34:42-67.

[4] See *Id.*

[5] See '933 Patent, Col. 29-32.

therapeutically useful quantities are not limited to cells having an "exogenous" EPO DNA sequence.

16. Dr. Lin's patent clearly contemplated use of DNA sequences more broadly than just "exogenous" cloned EPO DNA but also "endogenous" EPO DNA:

> "Novel DNA sequences of the invention include ***all sequences useful in securing expression in procaryotic or eucaryotic host cells*** of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of erythropoietin which are comprehended by: (a) the DNA sequences set out in FIGS. 5 and 6 herein or their complementary strands; . . . Specifically comprehended in part (b) are genomic DNA sequences encoding allelic variant forms of monkey and human erythropoietin. . ." ('933 Col. 11, lines 41-6.)

This passage makes clear to a molecular biologist at the time of invention that the DNA inventions were not limited to "cloned" versions, but to the "sequences," regardless of whether they are located in their native chromosomal locus or in "cloned" form.

17. Another relevant passage makes clear that Dr. Lin's inventions could be used to identify upstream DNA sequences within a human or other mammalian chromosome and to identify allelic variants:

> "DNA products of the invention may also . . . and employed in DNA hybridization processes to ***locate the erythropoietin gene position and/or the position of any related gene family in the human, monkey and other mammalian species chromosomal map. They can also be used for identifying the erythropoietin gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders.***" (Col. 12:12-21.)

18. For all these reasons, from the perspective of a molecular biologist at the time of invention, it would not have been scientifically reasonable to limit Dr. Lin's inventions to uses of a "cloned" human genomic EPO DNA sequence.

Opinion 2. Defendants' method of producing human EPO is neither "pioneering" nor "third-wave."

19. I understand that Defendants claim to have developed an entirely new way of making EPO and that they consider their method to be a "pioneering" "third-wave" development. Defendants' method is neither. It merely entails a logical use of established principles of recombinant DNA engineering long established by others.

20. Defendants' claimed distinctions from Dr. Lin's inventions, i.e. use of an "endogenous" EPO gene, use of "targeted" homologous recombination, use of "human" cells, and use of a promoter that is not "close" to the EPO gene, are all artifacts of their use of homologous recombination. But, homologous recombination alone cannot enable production of EPO. Without Dr. Lin's inventions, the tool of homologous recombination could not have been used to make therapeutically effective human EPO available to the world. Even homologous recombination combined with the prior art knowledge of human EPO could not have enabled a molecular biologist to produce human EPO. Only with the advent of Dr. Lin's inventions could homologous recombination be used in a process for making EPO. A molecular biologist would understand that homologous recombination, as used by Defendants, is merely ancillary to practicing what Dr. Lin taught — making human EPO available as a therapeutic.

21. Defendants' method is largely derivative. For example, Defendants did not invent a method of inserting a viral promoter upstream of a chromosomal human gene in a human cell using homologous recombination to achieve high level expression of the gene. Other researchers developed such methods well before Defendants. U.S. Patent No. 5,272,071, entitled "Method for the Modification of the Expression Characteristics of an Endogenous Gene of a Given Cell

Line,"[6] contains claims to using homologous recombination to insert a viral promoter upstream of a chromosomal gene in a human cell to achieve expression of the gene of interest.[7] This patent illustrates how little Defendant's methods differ from what others had already invented.

22. Defendants claim to have developed a "third-wave" "pioneering" invention distinct from a "second-wave" of recombinant DNA inventions. In effect, they argue that their use of homologous recombination to produce proteins in mammalian cells in culture renders the entire field of expressing proteins from cloned DNA sequences obsolete. Because Defendants' cell lines are recombinant cells expressing recombinant proteins and their derivative methods merely incorporate the tool of homologous recombination, I do not consider Defendant's methods or product to be "third-wave" or "pioneering."

Opinion 3. Defendants could not have achieved expression of human EPO in human cells without using Dr. Lin's inventions.

23. To develop their cell line for producing human EPO, Defendants critically depended on use of Amgen's human genomic EPO DNA sequence, the protein sequence, and many other Amgen inventions.

24. Based on my review of TKT's U.S. Patent No. 5,641,670 (the '670 Patent)[8] and Defendants' IND,[9] it is clear that Defendants relied heavily on Dr. Lin's teachings. Following is a listing of Dr. Lin's information that Defendants used and how they used it:

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[6] Exh. E.

[7] Claim 1 of US Pat. No. 5,272,071 provides: A method of activating a predetermined normally transcriptionally silent gene within the genome of a cell line so as to enable said cell line to express the gene product of said gene, comprising inserting a DNA construct into said genome by homologous recombination, said DNA construct comprising a DNA regulatory segment capable of stimulating expression of said gene when operatively linked thereto and a DNA targeting segment homologous to a region of said genome within or proximal to said gene, wherein said construct is inserted such that said regulatory segment is operatively linked to said gene of interest.

[8] See Exh. D, at Col. 26:62-Col. 27:45.

[9] Exh. E.

- Defendants used Dr. Lin's methods for amplifying a human EPO gene in cells to produce large quantities of human EPO.
- Defendants used Dr. Lin's vertebrate cell inventions, e.g., created a mammalian cell containing a recombinant DNA containing a mouse DHFR gene, a viral promoter, and an amplified EPO DNA sequence, to obtain high expression levels of human EPO.
- Defendants synthesized probes containing part of the Figure 6 sequence to isolate and characterize human genomic DNA clones containing the EPO gene locus. These isolated clones were used to create their recombinant DNA targeting construct.[10]
- Defendants used Dr. Lin's human EPO gene sequence to design their targeting construct to recombine into the chromosome upstream of the EPO gene.
- Defendants used the length, sequence and structure of the EPO signal peptide and the sequence and structure of the human EPO first exon/first intron/second exon regions to create an artificial first exon to replace the natural human genomic EPO first exon.
- Defendants used a sequence from Dr. Lin's Figure 6 sequence, the 5' splice donor site from the first EPO exon in their targeting construct, which splice donor site Defendants acknowledge is critical to their homologous recombination method.[11]

This extensive listing demonstrates that Defendant's could not have expressed human EPO in recombinant cells without using Dr. Lin's inventions. Moreover, it illustrates the striking similarities between Defendant's methods and Dr. Lin's.

25. The fact that Dr. Lin's recombinant DNA integrated randomly into the

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[10] See Example 1.f of '670 Patent, col. 26:61-66. (TKT 0067657-658) (Exh. D).

chromosome and Defendants' recombinant DNA integrated at a specific site in the chromosome is not scientifically significant. Both recombinant DNAs are amplified upon MTX selection, resulting in amplified copies of the "endogenous" human EPO gene and increased expression of EPO from that gene.

26. There is no discernible scientific benefit to Defendants' minor variations on Amgen's inventions. Whether one uses human cells or other mammalian cells, "endogenous" or "exogenous" EPO DNA sequences, targeted or random integration of DNA sequences into the cellular chromosome, or a promoter immediately adjacent to or at a distance from the EPO coding sequence, I am not aware of any advantages or distinguishing features of the resulting human EPO for pharmaceutical use.

Opinion 4. A molecular biologist at the time of invention could have made and used Dr. Lin's inventions with routine experimentation.

27. Having reviewed the Patent's specification and the Claims at Issue, it is clear to me, as a molecular biologist at the time of invention, that Dr. Lin described numerous inventions in sufficient detail that a molecular biologist could readily have practiced his inventions in many different ways. This specification does not contain simply a report of a "cloned" piece of DNA that appears to encode the human EPO gene. Instead, this specification contains a tremendous amount of detailed scientific research that makes available to molecular biologists all that was needed to make and use therapeutic human EPO using many different approaches.

28. For example, the patent specification describes in great detail both how Dr. Lin accomplished his many inventions, and all the specific information that a molecular biologist would have needed to reproduce and practice his inventions. Such information included:

[11] See Exh. D, '670 Patent, TKT 67662.

- methods of producing pharmaceutical human EPO,
- cells capable of producing relatively large quantities of therapeutic human EPO,
- the protein sequence of human EPO,
- the protein sequence of the leader peptide of human EPO,
- the detailed sequence and structural organization of the natural, genomic human EPO gene in Figure 6, including the coding sequences, the location and sequence of the introns and exons, the location and sequence of the leader peptide, the promoter, and 3' sequences.

The knowledge of all of this detailed information unlocked and enabled a myriad of applications of Dr. Lin's inventions. A molecular biologist would have been able to apply these teachings to make therapeutically effective human EPO using different mammalian cells, different promoters, and many other variations, with routine experimentation.

29. Confirming the breadth and enduring value of Dr. Lin's patent disclosure is the fact that numerous researchers, including Defendants, have used Dr. Lin's inventions to make human EPO.[12] These researchers used the teachings of Dr. Lin's patent to make and use the subject matter of the Claims at Issue, including human EPO, methods of making human EPO, and cells capable of producing large quantities of human EPO.

30. Defendants' own work, detailed above, required use of Dr. Lin's inventions and patent disclosure. Their method of producing human EPO further confirms the breakthrough significance of Dr. Lin's patents, which enabled Defendants to make therapeutic human EPO.

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intron 1 of the human EPO gene. See Exh. E, at TKT 44215, TKT 44217.

[12] See Yanagi, H. et al., DNA 1989 Jul-Aug;8(6):419-27 (Exh. H); Powell, JS et al., Proc Natl Acad Sci U S A 1986 Sep;83(17):6465-9 (Exh. I).

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct to the best of my knowledge.

Dated: December 7, 1999

By: _____
Richard D. Kolodner, Ph.D.